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(54) Title: ASSAY OF FREE AND COMPLEXED PROSTATE-SPECIFIC ANTIGEN (PSA)

(57) Abstract

According to the method of the invention, immunoassays are applied to measure free PSA as well as a proteinase inhibitor complex. Free PSA and PSA complex are according to the invention measured by a non-competitive immunoassay employing at least two different monoclonal antibodies. The invention is further characterized by that the PSA proteinase inhibitor complex of interest is formed either with α_1-antichymotrypsin, α_1-protease inhibitor (API) or α_2-macroglobulin. Moreover, the invention is characterized by that free PSA, the PSA-proteinase inhibitor complex and their ratio are applied in the diagnosis of patients with prostate cancer.

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Assay of Free and Complexed Prostate-Specific Antigen (PSA)

The present invention relates to an immunoassay of prostate-specific antigen (PSA), in which specific reagent materials (antibodies) are used that allow the measurement of free PSA as well as the PSA proteinase inhibitor complex.

It also relates to the use of free PSA and the PSA proteinase inhibitor complex and their ratio as a useful marker in diagnosis of patients with prostate cancer.

BACKGROUND OF THE INVENTION

The prostate specific antigen (PSA) was first purified from prostatic tissue (Wang et al. Invest Urol 1979), but the same protein was almost simultaneously and indipendently characterized in the seminal plasma (Hara et al. J Lab Clin Med 1989; Graves et al. N Engl J Med 1985). PSA is now known to be a 33-kDa glycosylated single chain serine protease (Lilja, J Clin Invest 1985; Watt et al. Proc Natl Acad Sci (USA) 1986). The 237 amino-acid polyeptide backbone has extensive similarities with that of the glandular kallikreins (Lundwall et al. FEBS Lett 1987; Schaller et al. Eur J Biochem 1987). Unlike the trypsin-like glandular kallikreins, which display Arg-restricted substrate specifity (MacDonald et al. Biochem J 1988), PSA displays chymotrypsin-like substrate specificity (Akiyama et al. FEBS Lett 1987; Christensson et al. Manuscript 1990; Lilja et al. J Biol Chem 1989). PSA has been predicted to be produced as a presumably inactive zymogen (Lundwall et al. FEBS Lett 1987). Active PSA is secreted into the seminal plasma (Lilja, J Clin Invest 1985) where it is one of the most abundant proteins of the prostate (Lilja et al. The Prostate 1988; Dubé et al. J Androl 1987). The biological activity of PSA in semen relates to its limited proteolytic fragmentation of the predominant proteins

secreted by th seminal vesicl s (Lilja, J Clin Invest 1985; Lilja et al. J Clin Inv st 1987; McGee et al. Biol Reprod 1988).

Secondary to the release from the prostate epithelium PSA may also be detected in the circulation (Papsidero et al. Cancer Res 1980). Measurements of the serum concentration of PSA have now found widespread use in monitoring of patients with prostate cancer, although increased serum concentrations of PSA have also been reported in benign prostatic hyperplasia and secondary to surgical trauma of the prostate (Duffy, Ann Clin Biochem 1989; Brawer et al. Urology suppl 1989). However, it is presently unknown whether the immunoreactivity in serum represents the PSA-zymogen, the active PSA or PSA inactivated by extracellular proteinase inhibitors and contradictory results have been reported on the molecular mass of this immunoreactivity. Papsidero reported in 1980 the PSA-immunoreactivity to elute as a single 90 to 100 kDa peak (Papsidero et al. Cancer Res 1980), whereas Alfthan and Stenman reported the predominant part of this immunoreactivity to elute as a 30-kDa protein (Alfthan et al. Clin Chem 1988) when subjected to gel filtration chromatography.

In the proceeding invention we showed that PSA has the ability to form complexes with proteinase inhibitors that occur in high concentration in the human extracellular fluids and that PSA occurs in these fluids both in a free and complexed form. In addition, the invention proved to be very useful in diagnosis of prostate cancer patients.

SUMMARY OF THE INVENTION

According to the method of the invention, immunoassays are applied to measure fr e PSA as well as PSA as a proteinase inhibitor complex. Fr PSA and th PSA compl x ar according to the invention measured by a non-comp titiv

immunoassay employing at 1 ast two diff rent mon clonal antibodi s. Th inv ntion is further characterized by that th PSA prot inase inhibitor complex of int rest is formed either with α_1-antichymotrypsin, α_1-protease inhibitor (API) or α_2-macroglobulin. Moreover, the invention is characterized by that free PSA, the PSA-proteinase inhibitor complex and their ratio are applied in the diagnosis of patients with prostate cancer.

DESCRIPTION OF THE DRAWINGS

FIG. 1 presents a polyclonal antibody and three monoclonal antibodies used to probe proteins blotted onto PVDF-membranes after agarose gel electrophoresis. In lane 1 is 1 µg PSA, in lane 2 is 1µg PSA incubated at 37 C° for 30 min with 6µg of α_1-antichymotrypsin, and in lane 3 is 6 µg of α_1-antichymotrypsin.

FIG. 2 presents a polyclonal and three monoclonal antibodies used to probe proteins blotted onto PVDF-membranes after SDS-PAGE. In lane 1 is 1 µg PSA, in lane 2 is 1µg PSA incubated at 37 C for 30 min with 6 µg of α_1-antichymotrypsin, and in lane 3 is 6 µg of α_1-antichymotrypsin.

FIG. 3 presents the specificity of the three assay versions.

FIG. 4 presents a correlation of PSA immunoreactivity in serum samples from 65 individual patients when analyzed with assay versions A and C.

FIG. 5 presents a correlation of PSA immunoreactivity in serum sampl s from 65 individual patients wh n analyzed with assay versions A and B.

FIG. 6 pr sents a gel filtration of a patient sampl B on a TSK 250 HPLC column. The PSA immunoreactivity of the eluted fractions were analyzed by assay versions A, B and C.

FIG. 7 presents the a filtration of a patient sample C on a TSK 250 HPLC column. The PSA immunoreactivity of the eluted fractions were analyzed by assay versions A, B and C.

FIG. 8 presents a gel filtration of a patient sample D on a TSK 250 HPLC column. The PSA immunoreactivity of the eluted fractions were analyzed by assay versions A, B and C.

FIG. 9 presents a characterization of PSA immunoreactivity in a serum sample with a PSA level of 10000 µg/l by gel filtration. PSA and the PSA-ACT complex were measured by IFMA.

FIG. 10 presents the proportion of the PSA-ACT complex of the total PSA immunoreactivity in sera of patients with prostatic cancer as a function of the PSA concentration. The level of PSA was measured by IRMA and that of PSA-ACT by IFMA.

FIG. 11 presents the concentration of the PSA-API complex measured by IFMA as a function of the PSA concentration measured by IRMA in sera from patients with prostatic cancer.

DETAILED DESCRIPTION

1. Production and characterization of monoclonal antibodies

Production of anti-PSA sp cific monoclonal antibodies

Balb/c mice were immunized by intraperitoneous injections with 70 µg of PSA emulsified in equal volymes with Freund's complete adjuvant. The immunization was repeated after 3, 6 and 9 weeks with 50 µg of PSA emulsified with Freund's incomplete adjuvant. Three weeks later the mice were given a final booster with 40 µg of PSA and the mice were killed four days later. Lymphoid cells of the spleen were prepared and mixed in a 1:1 ratio with plasmacytoma cells (NS-1). The cells were fused and harvested in microtiter wells in KC-2000 (Hazleton Biologics Inc., Le nexa, USA) containing 200 g/L foetal calf serum and HAT-supplement H-0262 (1:50, Sigma) (Matikainen et al. J Gen Microbiol 1983).

Anti-PSA specific antibody production by the master clones was assayed with well strip plates coated with rabbit anti-mouse IgG (Lövgren et al. Talanta 1984). The strips were incubated with either the hybridoma supernatants or the standard (monoclonal antibody against PSA; 0812 Hybritech), washed, incubated with Eu-labelled PSA (50 ng per well), and the amount of bound Eu-labelled PSA was determined.

Cloning of the master clones by limited dilutions was performed as described (Staszewski and Yale, J Biol Med 1984). The desired clones were expanded intraperitoneally in Balb/c mice; the ascitic fluid being collected in 10 days. The IgG-fraction of the ascitic fluid was purified by chromatography on protein A-Sepharose following the protocol recommended by the manufacturer.

Solid phase bound PSA was used to test if one unlabelled monoclonal antibody could block the binding of another Eu-labelled anti-PSA MAb to the solid-phase bound PSA. The solid-phase bound PSA was obtained by the incubation of 25 µl aliquots of purified PSA (25 µg/L) and 200 µL of Assay-buffer DELFIA^{R} (50 mmol/L Tris, pH 7.75, 0.15 mol/L NaCl, 0.5 g/L BSA, and 0.5 g/L NaN_3) for 2 h in well strip plates coated with the 2E9 or the 5A10 anti-PSA MAb. The strips were washed and then incubated for 1 h with 200 µL of one

unlabelled anti-PSA MAb (0.005 - 50 µg/L). Again, the strips wer wash d, incubated for 1 h with another Eu-labelled anti-PSA MAb and the amount of bound Eu-labell d anti-PSA was determined.

Parial characterization of the epitope specificity of three monoclonal antibodies against PSA

Several clones produced monoclonal antibodies against PSA as indicated by fluorometric assay. Three of these (designated 2E9, 2H11 and 5A10) were expanded and the antibodies isolated from the ascitic fluid. The three monoclonal antibodies against PSA were used to probe proteins blotted onto PVDF-membranes after agarose gel electrophoresis (Fig. 1) or SDS-PAGE (Fig. 2) of 1 µg of PSA (lane 1); 1 µg of PSA incubated at 37°C for 30 min with 6 µg of α_1-antichymotrypsin (lane 2); and 6 µg of α_1-antichymotrypsin (lane 3). PSA blotted to PVDF-membranes from the agarose gels was identified by all three monoclonal antibodies whereas the PSA complexed to α_1-antichymotrypsin was identified by the 2E9 and the 2H11 antibodies but not by the 5A10 antibody. The 2E9 antibody was the only anti-PSA MAb that readily identified PSA and PSA complexed to α_1-antichymotrypsin when these proteins were blotted onto PVDF-membranes after SDS-PAGE. However, a minute reaction was also obtained with the PSA (but not with the PSA complexed to α_1-antichymotrypsin) when the 2H11 and the 5A10 antibodies were used to probe these proteins blotted onto PVDF-membranes after SDS-PAGE.

The epitope specificity of the three monoclonal antibodies was also characterized using three different sets of solid-phase sandwich assays. Thereby assay (A), where the 2E9 antibody was used as solid-phase catcher and Eu-labelled 2H11 was used as detecting antibody, displayed an almost identical dose-response for PSA as compared with PSA complexed to α_1-antichymotrypsin (Tabl 1; Fig. 3). This

contrasts with both assay (B), where th 5A10 antibody was used as catcher and Eu-labelled 2H11 was used as det cting antibody, which prefentially recognized PSA but only poorly recognized PSA complexed to α_1-antichymotrypsin, and with assay (C), where the 2E9 antibody was used as catcher and Eu-labelled antibody against α_1-antichymotrypsin was used as detecting antibody, which only recognized PSA complexed to α_1-antichymotrypsin (Table 1; Fig 3).

Solid-phase bound PSA was used to further characterize the epitope specificity of the three monoclonal antibodies against PSA; the solid-phase binding of PSA having been achieved by the use of well strip plates coated with the 2E9 or the 5A10 antibody. It was thereby found that none of the anti-PSA MAb's 2E9, 2H11 or the 5A10 significantly blocked the binding of each other when we tested the ability of one anti-PSA MAb to block the binding of another Eu-labelled anti-PSA MAb to the solid-phase bound PSA.

2. The occurance of PSA-proteinase inhibitor complexes in human serum

Analysis of PSA in human serum

Serum from individual patient samples (n = 65) were analyzed with the three different sets of assays (A, B and C). Regression analysis of the results obtained with assay A and assay C gave $y = 0.89x + 6.55$, $r = 0.97$ (Fig. 4); and the regression analysis between assay A and assay B gave $y = 0.10x + 9.56$, $r = 0.82$ (Fig. 5).

The total recovery of the immunoreactivity from the gel filtration experiments of patient samples on the TSK 250 HPLC column was equally high (82 to 107 %) with all three assay procedur s used (A, B and C). The gel filtration experiments of patient samples on the TSK 250 HPLC column showed that the predominant peak of PSA-immunoreactivity,

when analyzed with assay A, was id ntified in fractions eluting at a position corr sponding to a mol cular mass of 80 to 90 kDa while a minor peak of this immunoreactivity was found in fractions eluting at a position corresponding to a molecular mass of 25 to 40 kDa (Fig. 6-8). In much the same way, the analysis of the fractions eluted with assay C (specific for PSA complexed to α_1-antichymotrypsin) identified one predominant immunoreactive peak in the range 80 to 90 kDa (Fig. 6-8). However, when assay B was used to analyze the fractions eluted from the gel filtration experiments the predominant immunoreactive peak eluted at a position corresponding to mass of 25 to 40 kDa. The elution position of this peak corresponded to the minor immunoreactive peak identified with assay A (Fig. 6-8).

When serum samples from men with various levels of PSA (10 - 10 000 µg/L) were fractionated by gel filtration, two components corresponding to PSA and PSA-ACT were also observed. In samples with high PSA-levels the PSA-ACT complex dominated (Fig. 9). In female sera these components were not seen (not shown). The proportion of PSA-ACT of total PSA immunoreactivity increased with increasing PSA levels (Fig. 10). In sera from healthy males with PSA levels below 2.8 µg/L the proportion of PSA-ACT was 23 – 47 %, in samples with PSA levels of 2.8 - 10 µg/L the proportion was 26 - 86 % and in samples with higher levels the proportion increased further being 70 - 100 % at PSA levels over 1000 µg/L (Fig. 10).

On the basis of the concentrations of the complexes expressed in arbitrary units the main complex of PSA in sera was PSA-ACT complex. In samples with low PSA levels the concentration of both PSA-API and PSA-ACT were close to the detection limit. Therefore it was not possible to calculate the proportion of these complexes in normal samples. Cl arly elevated l vels of PSA-API complex occur d in samples with PSA lev ls over 40 µg/L and the levels t nd d to incr as with incr asing l vels of PSA (Fig. 11).

3. PSA and PSA-α_1-antichymotrypsin compl xes in the diagnosis of patients with prostate canc r

The three assay versions referred to under section "Characterization of the epitope specificity of three monoclonal antibodies against PSA" were used to test 144 patients with benign prostatic hyperplasia (BPH) and 122 patients with different stages of prostate cancer (CAP). The ratios between A: PSA complexed with α_1-antichymotrypsin/PSA total and B: PSA free non-complexed/PSA total were calculated as well as the clinical sensitivity and specificity for the measurement of total PSA and PSA α_1-antichymotrypsin alone (Table 2). It is obvious from the presented data that increased clinical specificity is achieved by measuring the PSA α_1-antichymotrypsin complex and that the ratios between PSA free/PSA total and PSA free/PSA complexed with α_1-antichymotrypsin are significantly different between BPH and CAP patients.

Table 1

The table 1 presents a dose-response of purified PSA and PSA complexed to α_1-antichymotrypsin when analyzed by three different sets of assays.

- The assay A is 2E9 anti-PSA MAb as solid phase catcher and Eu-labelled 2H11 anti-PSA MAb as detecting antibody.
- The assay B is 5A10 anti-PSA MAb as solid phase catcher and Eu-labelled 2H11 anti-PSA MAb as detecting antibody.
- The assay C is 2E9 anti-PSA MAb as solid phase catcher and Eu-lab ll d rabbit antibody against α_1-antichymotrypsin as detecting antibody.

Th columns 1 indicat th purified PSA and columns 2 indicat th PSA complexed to α_1-antichymotrypsin.

Tables 2a and 2b

The tables 2a and 2b present the results of the testing of the patient samples with three assay versions for free, complexed and total PSA. In the table BPH indicates benign prostatic hyperplasia, CAP indicates prostate cancer, G indicates the differentiatio n grade and T indicates the grade. The table 2b presents the sensitivity and the specifity.

Table 1.

	PSA assay					
PSA	A		B		C	
µg/L	1	2	1	2	1	2
1	6664	5250	6733	2119	435	4208
5	26897	23535	31487	3179	487	15662
10	53452	41064	65146	4573	559	30283
100	534860	460464	600057	33006	2105	267223
500	2231640	1826790	2631640	156712	12073	726596

1. Purified PSA; 2. PSA complexed to α_1-antichymotrypsin.

Table 2a.

		Correlation coefficient	Ratio mean
BPH (n=144)	A. PSA c/PSA tot	0.932	0.970
	B. PSA f/PSA tot	0.853	0.302
CAP (n=122)	A.	0.994	1.219
	B.	0.784	0.191
CAP, G1 (n=31)	A.	0.994	1.628
	B.	0.922	0.190
CAP, G2 (n=47)	A.	0.972	1.141
	B.	0.956	0.169
CAP, G3 (n=43)	A.	0.996	1.014
	B.	0.818	0.218
CAP T1-2 (n=56)	A.	0.985	1.044
	B.	0.868	0.178
CAP T3-4 (n=65)	A.	0.993	1.372
	B.	0.770	0.204
CAP T4 (n=25) (not treated)	A.	0.997	1.174
	B.	0.825	0.188
BPH (n=84) PSA≤5	A.	0.879	1.059
	B.	0.850	0.301
BPH (n=60) PSA>5	A.	0.888	0.846
	B.	0.735	0.303
CAP (n=26) PSA≤5	A.	0.913	1.773
	B.	0.826	0.202
CAP (n=94) PSA>5	A.	0.993	1.065
	B.	0.778	0.188
CAP (n=25) PSA>5≤20	A.	0.919	1.025
	B.	0.502	0.187
CAP (n=69) PSA>20	A.	0.993	1.080
	B.	0.770	0.184

Table 2b.

Sensitivity and specificity

PSA tot	Sensitivity	PSA tot	>5	95/121=0.785
			>10	80/121=0.661
	Specificity	PSA tot	<5	84/144=0.583
			<10	116/144=0.806
PSA c	Sensitivity	PSA c	≥5	93/121=0.769
			>10	81/121=0.669
	Specificity	PSA c	<5	92/144=0.639
			<10	124/144=0.861

CLAIMS

1. An immunoassay of prostat -specific antig n (PSA) characterized by that in the immunoassay of prostate-specific antigen (PSA)
- an amount of prostate-specific antigen complexed with a proteinase inhibitor and/or
- an amount of free non-complexed prostate-specific antigen and/or
- an amount of the total prostate-specific antigen

are measured.

2. An immunoassay according to claim 1 characterized by that
- the PSA-proteinase inhibitor complex (complexed PSA) and/or
- the free non-complexed prostate-specific antigen (free PSA) and/or
- the total prostate-specific antigen (total PSA)

are measured by a non-competitive immunoassay.

3. An immunoassay according to claim 2 characterized by that
- the PSA-proteinase inhibitor complex (complexed PSA) and/or
- the free non-complexed prostate-specific antigen (free PSA) and/or
- the total prostate-specific antigen (total PSA)

are measured by a non-competitive immunoassay employing at least two different monoclonal antibodies.

4. An immunoassay according to claim 3 characterized by that the monoclonal antibodies used for the recognition of the PSA-proteinase inhibitor complex (complexed PSA) bind either to
- prostate-sp cific antigen (PSA), or to
- the PSA-prot inase inhibitor complex (complexed PSA) or to

- th prot inas inhibitor.

5. An immunoassay according to claim 4 characterised by that the proteinase inhibitor is α_1-antichymotrypsin.

6. An immunoassay according to claim 4 characterized by that the proteinase inhibitor is α_1-proteinase inhibitor.

7. An immunoassay according to claim 4 characterized by that the proteinase inhibitor is α_2-macroglobulin.

8. An immunoassay according to claim 2 characterized by that the ratio between the free non-complexed prostate-specific antigen (free PSA) and the PSA-proteinase inhibitor complex (complexed PSA) is determined.

9. An immunoassay according to claim 2 characterized by that the ratio between the free non-complexed prostate-specific antigen (free PSA) and the total prostate-specific antigen (total PSA) is determined.

10. An immunoassay according to claim 2 characterized by that the ratio between the PSA-proteinase inhibitor complex (complexed PSA) and the total prostate-specific antigen (total PSA) is determined.

AMENDED CLAIMS

[received by the International Bureau on 24 December 1991 (24.12.91) ; original claims 1-10 replaced by amended claims 1-8 (3 pages)]

1. An immunoassay of prostate-specific antig n (PSA) characterized by that in the immunoassay of prostate-specific antigen (PSA)
- an amount of prostate-specific antigen complexed with a proteinase inhibitor, where the proteinase inhibitor is α_1-antichymotrypsin, and/or
- an amount of prostate-specific antigen complexed with a proteinase inhibitor, where the proteinase inhibitor is α_2-macroglobulin, and/or
- an amount of free non-complexed prostate-specific antigen and/or
- an amount of the total prostate-specific antigen as a sum of free and complexed prostate-specific antigen

are measured.

2. An immunoassay according to claim 1 characterized by that
- the PSA-proteinase inhibitor complex (complexed PSA), where the proteinase inhibitor is α_1-antichymotrypsin, and/or
- the PSA-proteinase inhibitor complex (complexed PSA), where the proteinase inhibitor is α_2-macroglobulin, and/or
- the free non-complexed prostate-specific antigen (free PSA) and/or
- the total prostate-specific antigen (total PSA) as a sum of free and complexed prostate-specific antigen

are measured by a non-competitive immunoassay.

3. An immunoassay according to claim 2 characterized by that
- the PSA-proteinase inhibitor complex (complexed PSA), where the proteinase inhibitor is α_1-antichymotrypsin, and/or

- the PSA-proteinase inhibitor complex (complexed PSA), where the proteinas inhibitor is α_2-macroglobulin, and/or

- the free non-complexed prostat -specific antigen (free PSA) and/or
- the total prostate-specific antigen (total PSA) as a sum of free and complexed prostate-specific antigen

are measured by a non-competitive immunoassay employing at least two different monoclonal antibodies.

4. An immunoassay according to claim 3 characterized by that the monoclonal antibodies used for the recognition of the PSA-proteinase inhibitor complex (complexed PSA) bind either to

- prostate-specific antigen (PSA), or to
- the PSA-proteinase inhibitor complex (complexed PSA) or to
- the proteinase inhibitor.

5. An immunoassay according to claim 3 characterized by that the monoclonal antibodies used for recognition of free non-complexed prostate specific antigen (free PSA) bind to -prostate specific antigen, and
-that at least one of the epitopes defined by the monoclonal antibodies is exposed (available) only when the prostate specific antigen is not in complex with α_1-antichymotrypsin.

6. An immunoassay according to claim 2 characterized by that the ratio between the free non-complexed prostate-specific antigen (free PSA) and the PSA-proteinase inhibitor complex (complexed PSA) is determined.

7. An immunoassay according to claim 2 characterized by that the ratio between the free non-complexed prostate-specific antigen (free PSA) and the total prostate-specific antigen (total PSA) is determined.

8. An immunoassay according to claim 2 characterized by that the ratio between th PSA-proteinase inhibitor complex (complexed PSA) and the total prostate-specific antigen (total PSA) is determined.

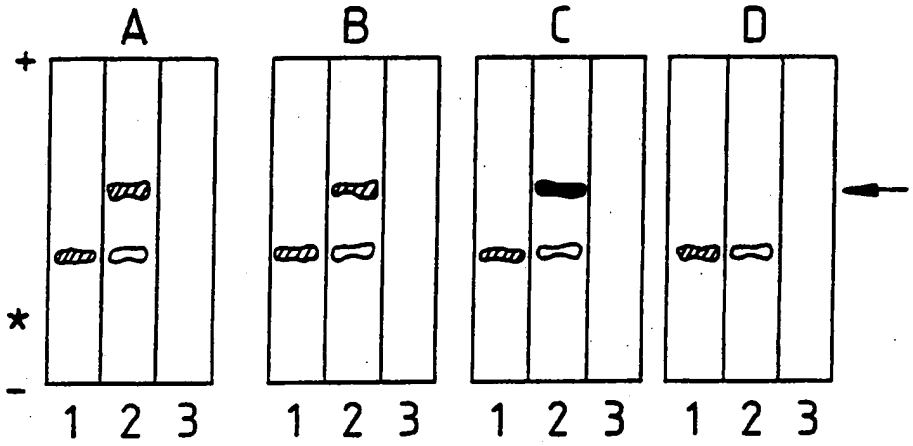


FIG. 1

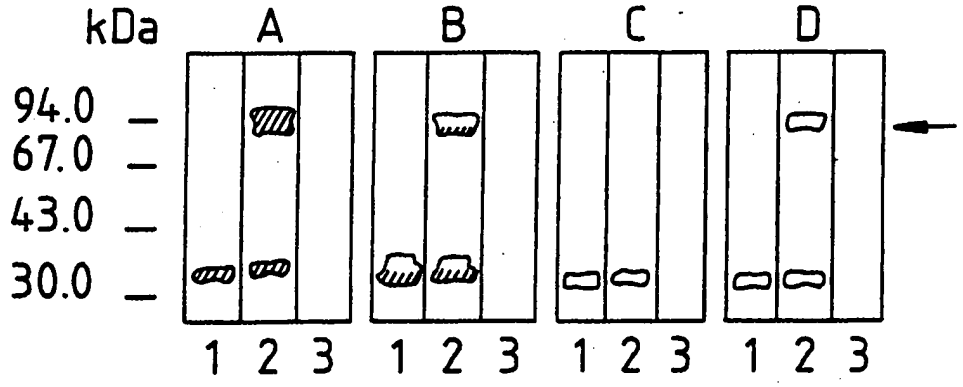


FIG. 2

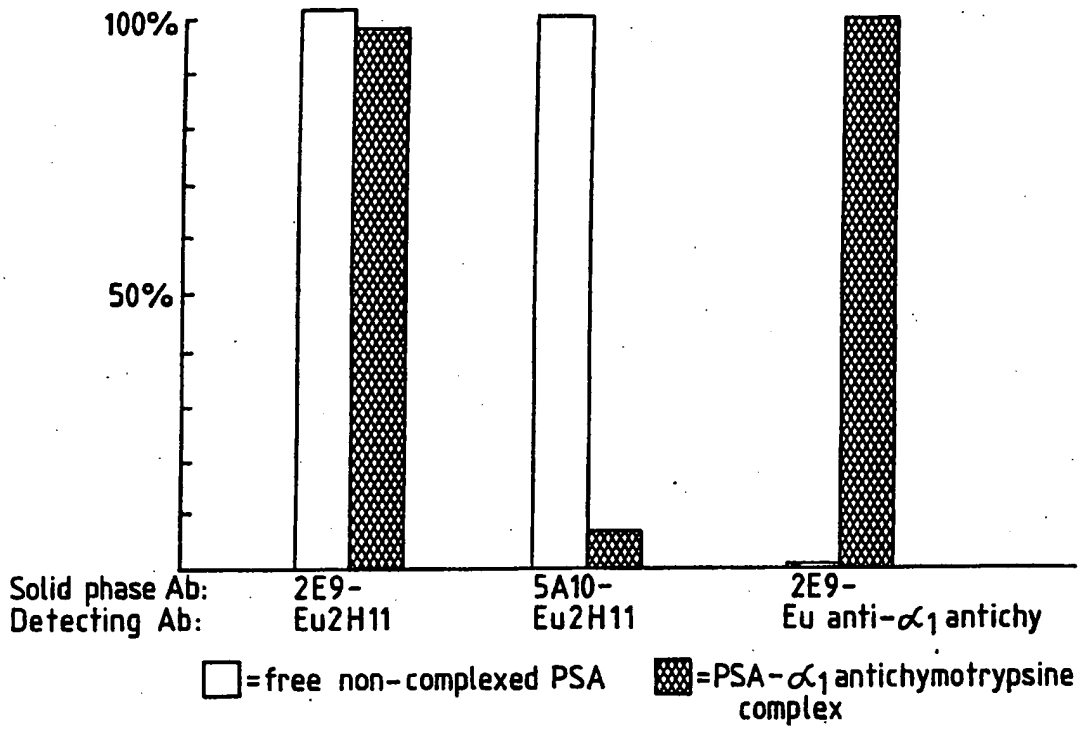
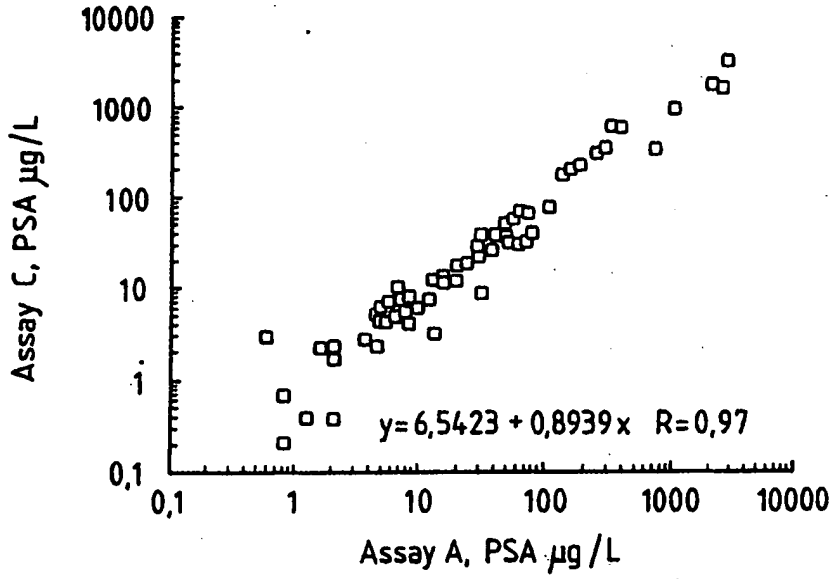

100%
50%
Solid phase Ab:
Detecting Ab:
2E9-
Eu2H11
5A10-
Eu2H11
2E9-
Eu anti-α1 antichy
=free non-complexed PSA
=PSA-α1 antichymotrypsine complex

FIG. 3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100
10
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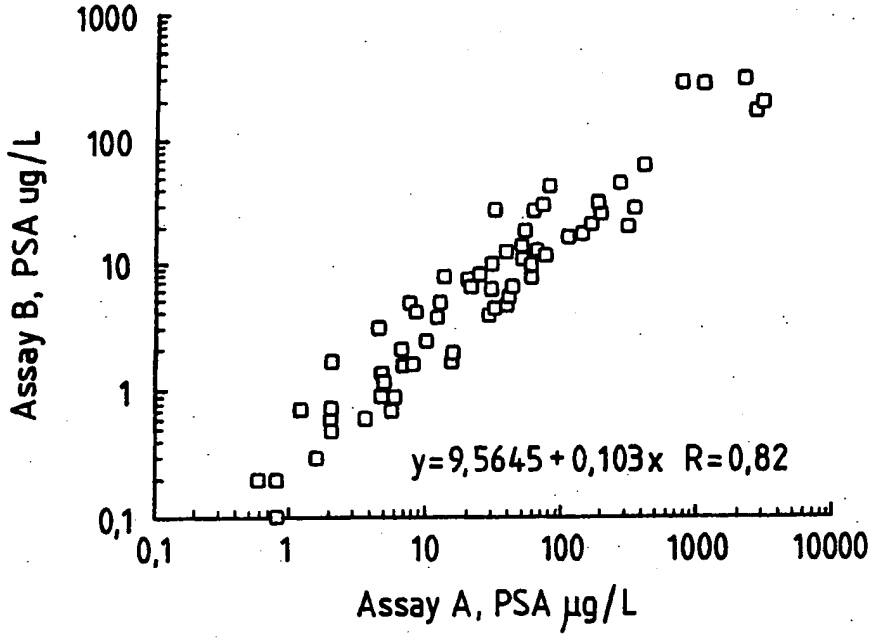
Assay A, PSA µg/L

FIG. 4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0,1
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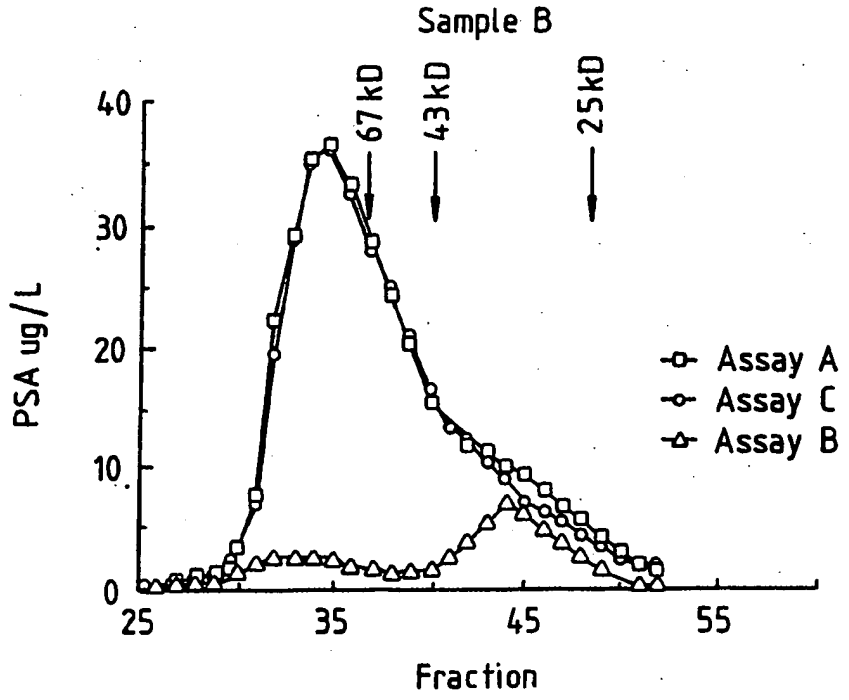
Assay A, PSA µg/L

FIG. 5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30
20
10
0
PSA ug/L
25
35
45
55
Fraction
Assay A
Assay C
Assay B

FIG. 6

Sample C

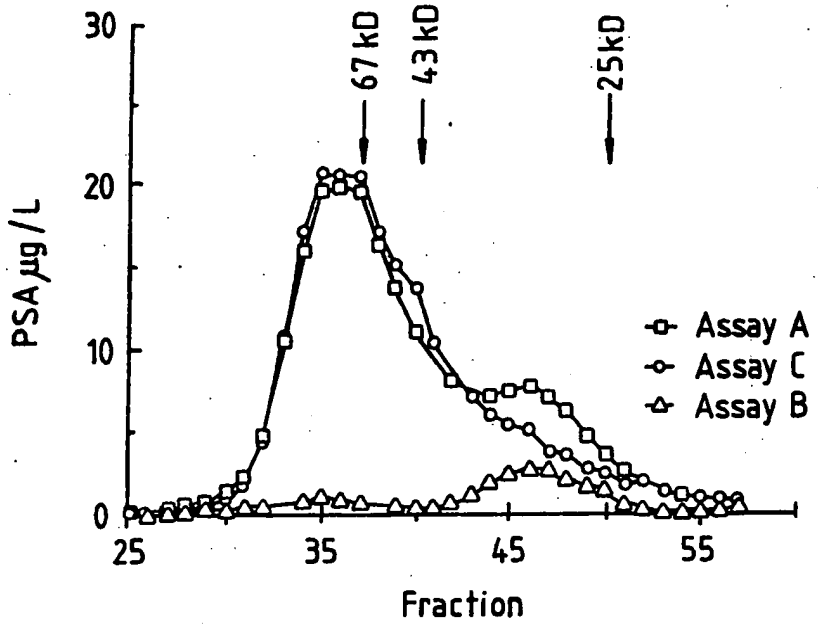

FIG. 7

Sample D

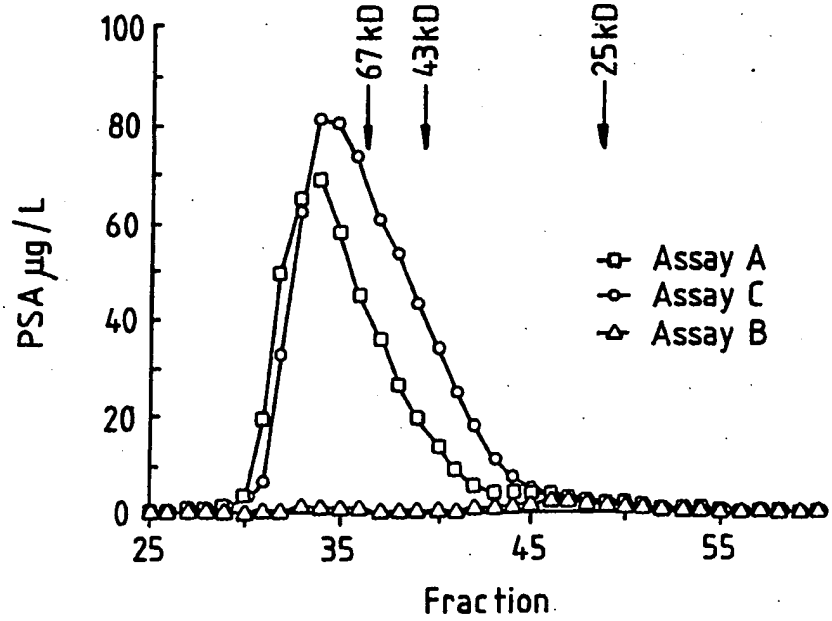

FIG. 8

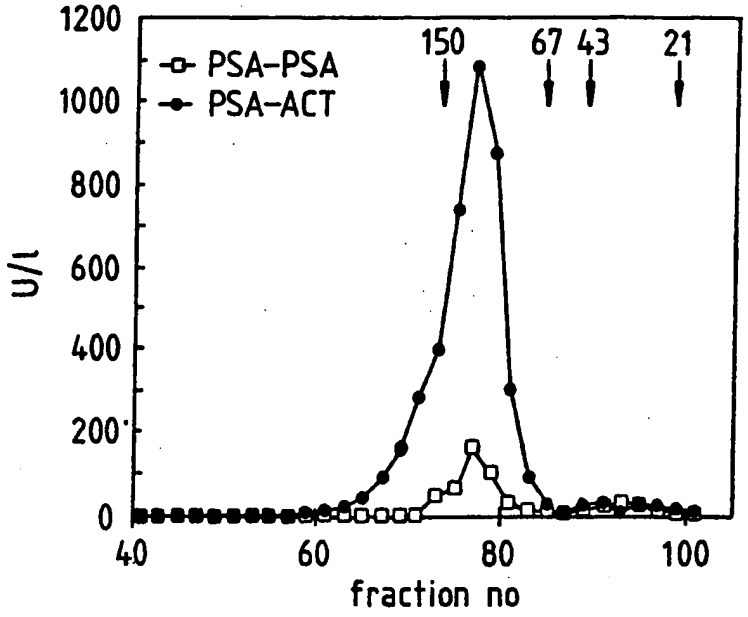
1200
1000
800
600
400
200
0
U/l
PSA-PSA
PSA-ACT
150
67
43
21
40
60
80
100
fraction no

FIG. 9

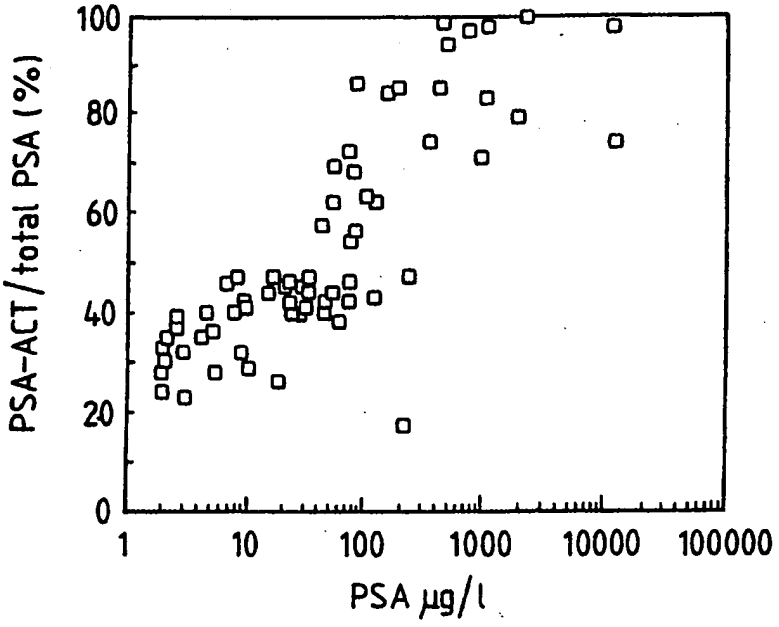
100
80
60
40
20
0
PSA-ACT/total PSA (%)
1
10
100
1000
10000
100000
PSA µg/l

FIG. 10

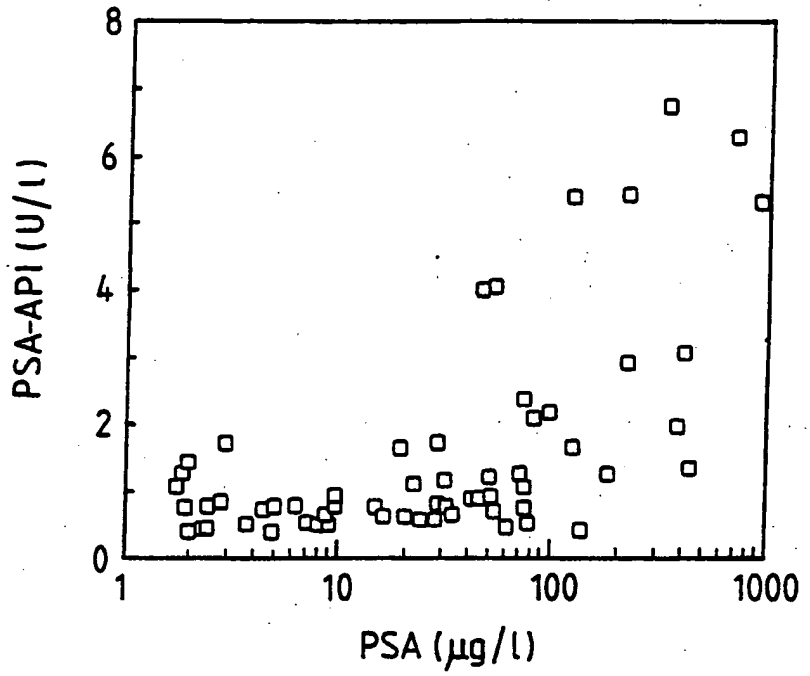

FIG. 11

INTERNATIONAL SEARCH REPORT

International Application No PCT/FI 91/00223

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 33/573, 574

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP, A1, 0160228 (CETUS CORPORATION) 6 November 1985, see the whole document	1-3
A	--	4-7
X	New York academy of sciences. N.Y. Annals., Vol. 417, 1983 T.M. Chu et al.: "Circulating antibody to prostate antigen in patients with prostatic cancer", see page 383 - page 389	1-3
	--	
X	US, A, 4446122 (TSANN M. CHU ET AL.) 1 May 1984, see column 6, line 62 - column 7, line 5; column 19, line 1 - line 28 example 24	1-3
A	--	4-10

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24th October 1991	1991 -10- 28
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Carl Olof Gustafsson

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	EP, A2, 0196845 (HYBRITECH INCORPORATED) 8 October 1986, see the whole document	4-10
X	--	1-3
X	Dialog Information Service, File 351, WPIL, accession no. 007662133, (WAKP) Wako Pure Chem Ind KK: "Determining antigen specific to human prostate gland - using combination of monoclonal and poly-clonal anti human prostate gland antibody in sandwich type immunoassay", & JP 63215967 A 880908 8842 (Basic)	1-3
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A	Patent Abstracts of Japan, Vol 11, No 233, P600, abstract of JP 62- 46263, publ 1987-02-28 Chugai Pharmaceut Co Ltd	1-7
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A	Proc. Natl. Acad. Sci., Vol. 83, May 1986 K W K Watt et al.: "Human prostate-specific antigen: Structural and functional similarity with serine proteases", see page 3166 - page 3170	4-10
	--	
A	J. Clin. Invest., Vol. 76, November 1985 H Lilja: "A Kallikrein-like Serine Protease in Prostatic Fluid Cleavesthe Predominant Seminal Vesicle Protein", see page 1899 - page 1903	1-10
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/FI 91/00223

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 91-09-27
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A1- 0160228	85-11-06	AU-B-	583818	89-05-11
		AU-D-	4084585	85-10-10
		CA-A-	1252388	89-04-11
		JP-A-	60228962	85-11-14
		US-A-	4690890	87-09-01
US-A- 4446122	84-05-01	CA-A-	1165685	84-04-17
		EP-A-	0042428	81-12-30
		JP-A-	2000497	90-01-05
		US-E-	RE33405	90-10-23
		WO-A-	81/01849	81-07-09
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		AU-D-	5482486	86-10-02
		CA-A-	1271420	90-07-10
		JP-A-	61274263	86-12-04